11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.001 Fixed-dose single-pill formulation of nebivolol plus hydrochlorothiazide and separated formulations in human subjects: a bioequivalence study. Iwamoto RD¹, Vespasiano CFP¹, Laurito TL¹, Moreno RA², Mendes GD³, De Nucci G¹ ¹Unicamp – Farmacologia, ²Galeno Research Unit, ³UniCastelo

Introduction: Systemic arterial hypertension is a chronic disease and a public health problem. Every year, the hypertension complications leads to 9.4 million deaths worldwide. Thus, an adequate control of blood pressure is essential to prevent future complications. The combination of nebivolol and hydrochlorothiazide is one of the many fixed-dose single-pill antihypertensive formulation available on the market. The aim of this study was to quantify nebivolol and hydrochlorothiazide in combined and separated formulations, in human plasma.

Methods: High performance liquid chromatography coupled to tandem mass spectrometry was employed to quantify the concentration of drugs in human plasma. Tested formulations were: Nebilet HCT™ (nebivolol + hydrochlorothiazide 12.5 mg tablet, tested formulation, manufactured by Menarini), Nebilet™ (nebivolol 5 mg tablet, reference formulation, manufactured by Menarini) and Clorana™ (hydrochlorothiazide 25 mg tablet, reference formulation, manufactured by Sanofi). Results: Before the start of the study, the protocol was approved by the Committee of Research Ethics of the Pontifical Catholic University of Campinas, SP, Brazil (approval number: 534.851). 21 men and 25 women completed the clinical study. All formulations were well tolerated. Physical examination, electrocardiogram and laboratory tests performed at the end of study showed no relevant alterations. Cₘₐₓ (ng/mL), Tₘₐₓ (h), AUC₀–ₚₐₚ (ng.h/mL), AUC₀–inf (ng.h/mL), Kₑ (h⁻¹) and T₁/₂ (h) for nebivolol were 4.08±2.27, 1.47±0.71, 25.64±33.68, 29.28±49.02, 0.05±0.01 and 13.26±7.18 (Nebilet HCT™ formulation); 3.92±1.70, 1.36±0.84, 22.65±19.47, 23.69±19.75, 0.06±0.02 and 11.52±3.02 (Nebilet™ formulation). For hydrochlorothiazide were 180.68±60.73, 2.12±0.86, 1157.49±353.88, 1192.95±361.67, 0.07±0.02 and 10.55±2.64 (Nebilet HCT™ formulation); 166.34±47.82, 2.35±0.86, 1125.79±341.08, 1165.04±358.90, 0.07±0.01 and 10.56±1.91 (Clorana™ formulation). Conclusion: Since the 90% CI for Cₘₐₓ, AUC₀–ₚₐₚ and AUC₀–inf individual test/reference ratios were within the 80-125% interval (FDA and ANVISA guidelines), we concluded that Nebilet HCT™ is bioequivalent to Nebilet™ and Clorana™. Keywords: nebivolol, hydrochlorothiazide, bioequivalence study, human plasma, LC-MS/MS. References: ANVISA. Resolução 899, 2003. Patel JR et al. Development and validation of bioanalytical method for simultaneous estimation of ramipril and hydrochlorothiazide in human plasma using liquid chromatography-tandem mass spectrometry. Journal of Chromatography, 970: 53-59, 2014. Ramakrishna NVS et al. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospay ionization tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis, 39: 1006, 2005. US-FDA. Guidance for industry bioanalytical method validation, 2001. World Health Organization. A global brief on hypertension, 2013. Financial Support: CAPES and Biolab Farmacêutica.
11.002 Toxicological evidences of methanolic extract from leaves of *Rubus imperialis* in DSS-induced colitis in mice and cytotoxic potential of its components niga-ichigoside F1, tormentic acid and 2β, 3β, 19α-trihydroxyursolic acid. Somensi LB\(^1\), da Silva LM\(^1\), Boeing T\(^1\), Niero R\(^1\), Andrade SF\(^1\) Univali – Ciências Farmacêuticas

**Introduction:** This study has investigated the effects of *Rubus imperialis*, a berry popularly known as “amora-branca”, in acute colitis in mice induced by intake of 3% dextran sulfate sodium (DSS) and the cytotoxic potential of the main components of extract: niga-ichigoside F1, tormentic acid and 2β, 3β, 19α-trihydroxyursolic acid. Method: Colitis was induced by intake of 3% DSS in drinking water of the mice during five days. In parallel, mice were treated orally with vehicle (water, 10 ml/kg), 5-aminosalicylic acid (100 mg/kg) or methanolic extract from leaves of *R. imperialis* (MERI, 100 mg/kg), once a day, during seven days. The evaluation of colitis was performed by the disease activity index (DAI) and colons samples were collected for histological, histochemical and biochemical analysis, after the treatment period (approval number in CEUA: 033/14p). **Results:** Unexpectedly, the administration of MERI exacerbated DSS-induced colitis, as indicated by DAI heightened, weight loss and increased histological colonic injury. MERI also decreased the colon mucin levels and increased colonic TNF content, when compared to vehicle-treated colitic mice. The colonic levels of reduced glutathione and the superoxide dismutase activity in colitic group treated with MERI decreased at similar levels to vehicle-treated colitic animals. Despite the worsening of colitis, MERI not altered the intestinal transit, body weight, colon length or organs weight in normal mice. In addition, no signs of cytotoxicity in L929 cells were verified after niga-ichigoside F1 incubation up to 100 µg/mL. However, the tormentic acid (100 µg/mL) and 2β, 3β, 19α-trihydroxyursolic acid (1 - 100 µg/mL) reduced the L929 cells viability. **Conclusion:** Thus, MERI may have aggravated the DSS-induced colitis phenotype through intense intestinal mucus barrier impairment, which would lead to inflammatory responses. Given the cytotoxic findings is probable that the torment acid and the 2β, 3β, 19α-trihydroxyursolic acid contribute to the intestinal damage verified in colitic mice after MERI treatment. **Financial support:** CNPQ, CAPES.
Introduction: The yerba-mate (Ilex paraguariensis A. St.-Hil.) is a native plant from South America regionally consumed. Different traditional non-alcoholic beverages (mate, mate tea, chimarrão, tereré) are obtained from the yerba-mate leaves. Mate is a rich source of bioactive phenolic compounds, mainly caffeoylquinic acids. The wealth of different mono and dicaffeoylquinic acids is a peculiarity of products derived from yerba-mate. To face the burden of cardiovascular diseases (CVD) a growing interest is assigned to the development of healthier foods, especially those naturally rich in bioactive phenolic compounds with protective effects against the development of chronic diseases. Different in vitro and animals studies associate the yerba-mate consumption with cardiovascular protection mechanisms. Consistent information about this activity in humans they are scarce and relating to your long-term consumption. The aim of this study was assess through a controlled trial the impact of a chronic intake of yerba-mate extract, rich in phenolic compounds, on intermediate biomarkers of cardiovascular health in humans. Methods: Yerba mate extract was obtained to DOMANI Co. - Pato Bragado / PR. The extraction process was performed for aqueous maceration. Determination of Total Phenols followed by Follin-Denis method using pyrogallic acid as standard. The study consists in controlled, randomized, double blind, crossover and phase 1 clinical trial. The study has a maximum duration of 84 days including the wash-out period. Twelve healthy male volunteers aged 45 to 65 were selected according to inclusion and exclusion criteria. The volunteers have to consume daily for 4 weeks the yerba-mate extract (700-750 mg of phenolic compounds / day) or placebo (PLB). At the beginning and at the end of each experimental period, blood is sampled for measurement of glycemic and lipid parameters, and inflammatory markers. Urine samples were collected for analysis to characterize the exposure profile of volunteers in response to yerba-mate phenolic compounds consumption. Results: Twelve volunteers completed the trial. The difference of the biochemical parameters after and before treatment were Total Cholesterol (EIP= 13.7±17.4 mg/dl, PLB= 0.7±21.7 mg/dL - P < 0.05); and HDL (EIP= 4.9±12.9 mg/dl; PLB= 1.5±8.9 mg/dL); LDL (EIP= 8.1±29.5 mg/dl, PLB= -0.3±19.7 mg/dl); Triglycerides (EIP= 12.0±93.6 mg/dl; PLB= -3.3±39.2 mg/dl) and plasma glucose (EIP= -23.3±36.9 mg/dl; PLB=-8.2±7.4 mg/dL) showed no significant difference. Yerba-mate extract attenuated or slowed the increase plasma glucose after consumption of glucose (75 mg) for eight of the twelve volunteers in the oral glucose tolerance test. Conclusion: The yerba-mate extract consumption was able to modify lipid and glycidic parameters in healthy volunteers, not significantly. In addition, more specific tests, such as dosage of inflammatory markers, cell adhesion markers and transcriptome will still be performed. National Research Ethics Committee - Ministry of Health/Brazil – n° 22531313.5.0000.0109 Acknowledgments: Centro Universitário da Grande Dourados (UNIGRAN) e Hospital Universitário da Grande Dourados the availability of space and collection and analysis.
11.004 Differential EDN2 expression induced by plasma from nonresponsive preeclamptic patients in endothelial cells. Dias MC, Cavalli RC, Deffune E, Sandrim VC

Introduction: Preeclampsia is characterized by hypertension and proteinuria at ≥ 20 weeks of gestation and it is the leading cause of fetal-maternal morbidity and mortality worldwide (Report of the National High Blood Pressure Education Program Working Group. Am J Obstet Gynecol, v.183, p.1, 2000; WHO. Am J Obstet Gynecol, v.158, p.80, 1988). Antihypertensive therapy is used to control blood pressure in preeclampsia, however most of the preeclamptic women do not respond to any antihypertensive drug, which increases the severity of the clinical outcome of these patients compared to those who are responsive (Sandrim, V.C. Pharmacogenomics J, v.10, n.1, p.40, 2010). Moreover, there are differences in the genetic profile between nonresponsive and responsive patients (Sandrim, V.C. Pharmacogenomics J, v.10, n.1, p.40, 2010). We examined the effects of plasma incubation from responsive (PER) and nonresponsive (PENR) preeclamptic patients on the expression of 84 genes related to endothelial cell biology using PCR-array in human umbilical vein endothelial cells (HUVECs). EDN2, gene encoding the vasoconstrictor peptide endothelin-2 (ET-2), was the most differently expressed gene, therefore we investigated ET-2 levels in plasmas and cell supernatants from PER and PENR. Methods: HUVECs were incubated with 20% (v/v) plasma from PER and PENR for 24h. PCR array was performed in the incubated HUVECs (n=4, per group) and ET-2 production was measured in plasma and the cell supernatants by ELISA (n=13, per group). Results: EDN2 was significantly upregulated in PENR compared to PER (fold change=19.01). ET-2 concentration was not significantly different between the PER and PENR in plasmas (0.119±0.005 pg/mL; 0.152±0.014 pg/mL, p=0.055, respectively) and cell supernatants (0.084±0.004 pg/mL; 0.086±0.004 pg/mL, p=0.827, respectively). Conclusions: EDN2 was significantly more expressed in the nonresponsive group, however ET-2 production was not different between the groups. It suggests that plasma from nonresponsive patients induces EDN2 expression, however a possible post transcriptional regulation mechanism, by microRNAs for example, might be occurring, which explains why ET-2 production is not increased. Financial Support: CNPq Research Ethical Committee (CEP) approval: process number 035/2009
11.005 Reproductive toxicity assessment of *Origanum majorana* essential oil in Wistar rats. Dantas AS¹, Santos LD¹, Mello FB², Mello JRB¹ UFRGS – Farmacologia e Toxicologia

**Introduction:** *Origanum majorana* L., a plant rich in essential oils, presents antifungal activity in vitro against Malassezia pachydermatis, Candida spp and Trichosporon asahii, among other activities. However, exposure to chemicals, including herbal medicines, can cause infertility, maternal toxicity, embryotoxic effects on offspring. Therefore, this study investigated in vivo reproductive toxicity of the *Origanum majorana* essential oil (Omeo) and major compounds on rats Wistar, effects on the fetal development and behavioral parameters of the offspring.

**Methods:** The study was performed with 240 sexually mature Wistar rats (n = 60 males and 180 females; 70 days old) supplied by the Center of Reproduction and Experimentation of Laboratory Animal, divided in 10 male and 30 female per group. The in vivo tested doses were 33mg/kg, 100mg/kg or 300mg/kg Omeo, 77mg/kg γ-terpinene or 52mg/kg terpinen-4-ol, established according to previous studies. Negative control group received the vehicle of the solution. Doses it was given by oral gavage (10ml/kg body weight) to male parents for 91 days and to females for 14 days prior to mating, during mating, pregnancy and lactation. Three virgin females were placed inside de cage of one male for 2h each day. Vaginal smears it was realized daily until sperm positive or “plug” presence observed. To identify alterations in reproductive system of males were evaluated organs relative weight, histology, sperm number and morphology of spermatozoa. Reproductive index, body weight gain, intake water and food, and toxicity signs were evaluated. Signs of maternal toxicity, maternal behavior, offspring reproductive parameters, open field behavior and sexual behavior were observed. Data are given as means and ANOVA or Chi-square test was applied to statistical analyses (P<0.05 = significantly different). **Results:** The most abundant compounds identified by gas chromatograph coupled to a mass spectrometer included γ-terpinene (25.73%) and terpinen-4-ol (17.24%). The exposure induced dose-dependent way reduction in reproductive index, reduction in sperm number, reduction in daily sperm production, increase in sperm defects to males treated with Omeo and Terpinen-4-ol, reduction in testis and epididymis relative weight to 300mg/kg Omeo, and no pregnancy at this dose and terpinen-4-ol. Maternal toxicity was not observed. The maternal behavior related to the nest was reduced with 100 mg/kg Omeo, but the maternal responsiveness was maintained by the nursing behavior. This dose induced reduction in the number of offspring per parent, increase in the time of pregnancy and changes in the development of males characterized by delayed preputial separation. Other reproductive and behavioral parameters of the male offspring were not affected. **Conclusion:** In conclusion, the continuous treatment with high doses Omeo and Terpinen-4-ol affect male fertility, induce changes in reproductive rates, cause adverse effects on the developing offspring, and should not be used during sexual age, pregnancy and lactation. **Financial support:** Conselho Nacional de Pesquisa (CNPq) and UFRGS PROPG. Work approved by CEUA UFRGS, Protocol number 23613.
Tramadol (TR) is an opioid analgesic widely used for acute and chronic pain and structurally related to codeine and morphine. TR possesses weak opioid agonist activity and inhibits serotonin and norepinephrine transport. It presents short duration of action and it is necessary repeated doses or continuous infusion for a prolonged analgesic action. TR may be valuable, when compared to other opioids, as it produces mild adverse effects such as nausea, dizziness, sedation, dry mouth and sweating. Due to these properties, TR was selected for the development of a new drug-delivery system formulation with thermoreversible poloxamers (PL). PL have the ability, in concentrated solutions, of forming gels close to corporal temperatures. This property can be used for parenteral administration of drugs and it prolongs their effects and/or allows equivalent effects to the commercially available formulations with lower doses. Therefore, the use of this new TR formulation might present improved pharmacokinetics (PK) and pharmacodynamics (PD) and it also may be an advantage for pain relief. Thus, this in vivo study evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of new formulations of tramadol (TR) in thermoreversible gels poloxamers (PL). In the PK assay, New Zealand albino rabbits were divided into five groups (n = 6) and treated subcutaneously (10µg.kg\(^{-1}\)) with the formulations: F1- TR 2%; F2 PL 407 (20%) + PL 188(10%) + TR 2%; F3-PL 407 (25%) + PL 188 (5%) TR + 2%; F4- PL 407 (18.5%) + PL 403 (1.5%) + TR 2%; F5- PL 407 (20%) + TR 2%. Blood samples were collected 0 (predose), 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes after administration. TR plasma levels were determined using a triple stage quadrupole mass spectrometer (LC-MS/MS), equipped with an API electrospay source. The PK parameters were calculated using WinNonlin software version 5.3 (Pharsight Corporation). Data were submitted to ANOVA/Kruskal-Wallis test (post-hoc). F2 showed higher plasma concentrations than F1 after 120 minutes (p<0.05), after 180 minutes the differences were among F2 and all the other formulations (p<0.05). F2 values for areas under the curve (ASC\(_{0-480}\) e ASC\(_{0-}\infty\)) presented differences between F1 and F5 (p<0.05). F2 clearance was smaller than F1 (p<0.05). The PD assay of the same formulations was performed with a Wistar rats postoperative pain model. The animals (n=6/group) received a hind paw incision and after 24h the formulations of TR were subcutaneously administrated (10µg.kg\(^{-1}\)). Mechanical hypersensitivity was measured using von Frey filaments after 15, 30, 60, 90, 120 and 180 minutes of TR formulations application. Data were submitted to ANOVA/ Tukey-kramer test (post-hoc). F2 produced more intense analgesia after 120 and 180 minutes when compared to the other formulations (p <0.05). Based on these results, we concluded that F2 provided efficient analgesia and it was effective to promote slow release of TR. Minami K. et al. μ-Opioid receptor activation by tramadol and O-desmethyltramadol (M1). J Anesth, 29 (2015), 475-9. Fisher M.et al. Int. J. Clin. Pharmacol. Ther, 48 (2010), 138-145. Schmolka IR. J. Biomed. Mater. Res. 6 (1972), 571-582. The protocol was submitted to the Research Ethics Committee of the USF (n. 002.04.2013) The authors thank FAPESP (# 2012 / 16822-7) for the financial support.
11.007 Effect of caffeine in adenosine receptors expression in inflammation induced by copper in zebrafish larvae. Cruz FF, Leite CE, Kist LW, Bogo MR, Bonan CD, Campos MM, Morrone FB PUCRS

Introduction: Zebrafish (Danio rerio) has been used in high-throughput screening for chemicals toxicity (Hill, Toxicol Sci., V. 86, P. 6, 2005). Copper is a heavy metal that cause cellular damage by the production of ROS (Valko, Chem Biol Interact., V. 160, P. 1, 2006), and previous studies demonstrated its effect in purinergic signaling (Rosenberg, Toxicology, V. 236, P. 132, 2007). Adenosine is a product from ATP hydrolysis, which activate four adenosine receptors: A1, A2A, A2B and A3, promoting protective effects in inflammation (Antonioli, Nat Rev Cancer, V. 13, P. 842, 2013). Caffeine exerts most of its actions by antagonizing adenosine receptors (Ohta and Sitkovsky, Curr Opin Pharmacol. V. 9, P. 501, 2009). The aim of this study was to evaluate the effects of caffeine in adenosine receptors expression on a copper-induced inflammation in zebrafish larvae. Methods: Survival Curve: Larvae mortality was verified 0, 2, 4, 8 and 24 h after copper and/or caffeine exposure (25 larvae per group, n=3). Gene expression analysis: Adenosine receptors described in zebrafish (A1, A2A1, A2A2 and A2B), TNF, COX-2 and IL-10 genes expression was determined by RT-qPCR after 4 and 24 h of exposure (20 larvae per group, n=5). Statistical Analysis: We used Kaplan-Meier method for the survival curve and One-way Analysis of Variance (ANOVA) followed by Tukey’s test in the RT-qPCR. Data were expressed as mean ± standard error and P<0.05 was considered significant. Results: After 24 h exposure, the treatment with 10 µM CuSO4 reduced the larvae survival to 60% when combined with 500 µM caffeine, and to 0% when associated with 1 mM caffeine. Based on these results, we tested the combination of 10 µM CuSO4 plus 500 µM caffeine to access inflammatory markers and adenosine receptors gene expression. There was a decrease of COX-2 expression in the group treated with copper associated to caffeine after 4 h (0.52 ± 0.03), and an increase after 24 h (1.07 ± 0.15). TNF expression was increased in 10 µM CuSO4 plus 500 µM caffeine treatment, after 4 and 24 h (4.69 ± 0.25; 4.76 ± 0.81, respectively). Larvae treated with 10 µM CuSO4 plus 500 µM caffeine had a decrease of IL-10 expression after 24 h (1.08 ± 0.06). At 4 h, 10 µM CuSO4, 500 µM caffeine and 10 µM CuSO4 plus 500 µM caffeine groups presented an increase of A1 (8.67 ± 0.27; 8.09 ± 0.67; 9.73 ± 0.86, respectively) and A2A2 (1.30 ± 0.02; 1.44 ± 0.09 and 1.52 ± 0.60, respectively) gene expression. 10 µM CuSO4 plus 500 µM caffeine increased the expression of A2B receptor (4.61 ± 0.39; 4.95 ± 0.46, respectively), at 4 h of exposure. 24 h treatment with 10 µM CuSO4 plus 500 µM caffeine caused an increase of A1, A2A2 and A2B genes expression (8.39 ± 2.2; 1.52 ± 0.07 and 4.95 ± 0.46, respectively). Conclusion: Our results demonstrate that caffeine can potentiate the inflammatory effect of copper involving the modulation of adenosine receptors. FINANCIAL SUPPORT: Capes, PUCRSINFRA, FAPERGS/CNPq n. 008/2009 (PRONEX). Institutional Animal Care Committee approval: 09/00135, CEUA–PUCRS.
Detection Of Adverse Cutaneous Drug Reactions – A Pharmacovigilance study

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Introduction: Pharmacovigilance is a science that has as its main objective the detection of adverse events and their subsequent notification. Nowadays, almost every day, a new drug enters market triggering an increase of drug commercialization along with an increase of the risk of adverse events. Adverse drug reactions (ADRs) are a frequent problem in medical practice, among which the most common are the adverse cutaneous drug reactions (ACDR). These ACDR constitute a major health problem as they cause an increment in morbidity and mortality and increase health costs. ACDR have a high frequency and may produce severe health damage thus it is important to make an early diagnosis. The objectives of this study were to determine the pharmacological groups involved in ACDR, its clinical presentation, and finally to establish causation. Methods: This study was descriptive, prospective and observational. It was carried out at three health institutions from Córdoba Province, Argentina: Hospital Italiano, Hospital Ferreyra and Hospital Privado. Reports of adverse events were recorded in the pharmacovigilance yellow cards provided by ANMAT and a specific tab designed was completed. The variables analyzed were: responsible drug for the adverse event, clinical presentation, severity of ADRs and causation. Results: From a total of 110 patients, antimicrobial drugs (34.5%) produced the most frequent ADRs, followed by Analgesic / Anti-inflammatory, and finally anticonvulsants. The most frequent form of clinical presentation was rash (55%), followed by urticaria and erythema. Causality was probable in 90% of patients, and defined (tested) in an 8.61%. According to the severity we registered: moderate in 81% of patients, severe in 13.63%, and minor in 5, 37%. Conclusion: Manage or prevent any case of ADRs and ACDR constitute a pillar for health care and to reduce health costs. In this work we observed that antibiotics and analgesics are among the most common used drug groups thus increases the probability of being the cause of most of the ADRs events. The rash was the clinical presentation more frequent in ACDR. According on the severity of clinical presentation, the minor forms do not disrupt the daily life of the patient so the hospital consultation is often low. However severe and moderate clinical presentations interrupt the daily activities and lead the patients to consult physicians. Therefore, physicians should be familiarized with these conditions and be prepared to diagnose an ADRs and to identify the corresponding ACDR.

Comité Internacional de Ética en Investigación en Salud –CIEIS n° 02/2013
INVESTIGATION OF THE ASSOCIATION OF CYP1A2*1C POLYMORPHISM WITH SUPER-REFRACTORY SCHIZOPHRENIA

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Introduction: CYP1A2-dependent metabolism of drugs has demonstrated that genetic polymorphisms can control enzyme activity. Among these variants, a G>A mutation at position -2964 in the flanking region of the CYP1A2 gene (CYP1A2*1C) causes a significant decrease in CYP1A2 inducibility (Hamdy et al. Br J Clin Pharmacol, 55, 321-324, 2002). CYP1A2 is the main enzyme involved in the clozapine (CLZ) metabolism. Knowing CLZ is an antipsychotic of choice for patients having treatment refractory schizophrenia (TRS) and that 30% of them do not fully respond to CLZ treatment, constituting the group having super-refractory schizophrenia (SRS), this study evaluated if CYP1A2*1C has association with SRS.

Methods: 28 patients who met the criteria for treatment-refractory schizophrenia (TRS) (responders to CLZ treatment) and 31 individuals with super-refractory schizophrenia (SRS) (non-responders to CLZ treatment) receiving CLZ at least 12 months were submitted to genotyping test. DNA of patients was extracted from venous blood and CYP1A2*1C polymorphism was analyzed by polymerase chain reaction (PCR) followed by sequencing technique. Genotype frequencies and demographic data were analyzed by comparison between groups (TRS and SRS) with X² and Fisher’s exact test using SPSS software package (version 21.0; SPSS Inc., IL, US). A p-value <0.05 was considered statistically significant.

Results: Similar genotype frequencies were found among TRS and SRS groups for wild-type homozygous (GG; 85.7 % and 61.3%, respectively) and the same frequencies for heterozygous (GA) and homozygous mutant (AA) (7.1%; and 19.1%, respectively) (P=0.05). Wild-type allele G showed frequencies of 89% (TRS) and 71% (SRS), while the mutant allele A was 11% (TRS) and 29% (SRS), respectively (P=0.03). Demographic data were not statistically different among TRS and SRS groups: age medium (41.32 ± 10.19 and 38.67 ± 9.23 years, P=0.09); male/female ratio (22/6 and 17/14 individuals, P=0.09); ethnic group white/black/brown (21/2/0 and 9/13/19 individuals, P=0.64); coffee consumption (21 and 17 individuals, P=0.17); smokers (14 and 12 individuals, P=0.54); body mass index (26.64 ± 0.82 and 28.03 ± 1.03 kg/m², P=0.30), respectively. Brief Psychiatric Rating Scale (BPRS) showed a high score for SRS (48.42 ± 2.36) when compared to TRS patients (32.00 ± 2.79) (P<0.0001), as well as the CLZ daily administered medium dose in SRS (606.5 ± 118.1 mg) in comparison with TRS (537.5 ± 114.4 mg) (P=0.03).

Conclusion: The results suggest the CYP1A2*1C polymorphism may be associated with SRS, since mutant allele frequency was increased in this patients group. Further studies using a larger group of individuals will be performed to confirm this finding.

Financial Support: CNPq, Capes, Fapeg.

Research approved by the Human Research Ethical Committee from Federal University of Goiás (protocol number 1.483.734).
EVALUATION OF PROSTATE PERMEABILITY OF TADALAFIL

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Introduction: Although Tadalafil has been indicated to treat lower urinary tract symptoms (LUTS), no studies have been developed to measure its concentration in prostate after treatment.

Aims: Due to the limitation of animal models to develop benign prostatic hyperplasia spontaneously, the morphology of monkey prostate be very similar to human prostate and a lack of studies related to tadalafil concentration on prostate, we propose a monkey model for evaluation of prostate permeability of tadalafil.

Methods: Tadalafil was single administered by intravenously (10mg/animal) or gavage route (10mg/animal) in male Callithrix jacchus (body weight: 250–360 g). After 2, 4 and 8 hours of oral treatment, the animals were anesthesized by association of ketamine (20mg/Kg) midazolan (2mg/Kg) and inhalation of isoflurane (5%) and samples of plasma and prostate were collected. Tadalafil levels were quantified by high performance liquid chromatography coupled to electrospray tandem mass spectometry. Macroscopic inspections were performed during the experiments. All protocols were approved by the Brazilian College for Animal Experimentation (COBEA; No 3811-1). The use of these animals was authorized by the Brazilian Institute of Environment (Sisbio:16951-1).

Results: Plasma and prostate tadalafil levels after two hours (intravenously administration) were 2928.03 ± 402.33 ng/ml and 70.28 ± 23.83 ng/mg (tissue), respectively (n=3). After two hours of a single oral administration were observed 567.65 ± 63.90 ng/ml of tadalafil on monkey plasma and 1.60 ± 0.31 ng/mg (tissue) on prostate (n=3). Plasma tadalafil concentration after four and eight hours of oral treatment were 262.64 ± 16.55 ng/ml and 246.89ng/ml ± 85.40, respectively (n=3 for each group). Prostate tadalafil contents were 17.84 ± 3.22 and 10.25 ± 4.96, consecutively. No side effects were observed during the protocols.

Conclusion: Tadalafil was evaluated in a reliable prostate permeability model by a specific and sensitivity analytical method.

Sources of Research Support: This study was supported by CNPq/Brazil
MONTECARLO SIMULATIONS TO PREDICT THE OUTCOME OF MENINGITES TREATMENT ASSOCIATED TO CRIPTOCOCCUS NEOFORMANS

Introduction: Meningitis caused by Cryptococcus neoformans is an important disease associated to high levels of mortality. The drug therapy usually involve Amphotericin B or azoles, which are more safety drugs. In the present study we applied the microdialysis as a tool to determine the free levels reached by the azoles (voriconazole (VRC) and fluconazole (FLU)) during an experimental disseminative criptococosis infection in rats and in also in healthy animals. The relationship between free cerebral/free plasma concentrations (Ft) in both conditions were evaluated and the concentration versus time profiles were analyzed by a population pharmacokinetics approach (POPPK), using Monolix. To compare the outcomes obtained after the treatment with both drugs using the levels reached in the infected and non-infected brain's Montecarlo (MC) simulations were done in 1000 combinations, taking into account the variability observed for the tissue levels reached by the drugs using Berkeley Madonna. Methods: The infection was induced by the i.v. administration of 100µL of inoculum (1·10⁵ CFU). The animals (n=6/group) were submitted to surgery to insertion a MD probe (CMA® 12) into the brain tissue in the region of the motor cortex (A:2.2mm; L:2.8mm; P:2.6mm). The doses administered was 5mg/kg i.v. of VRC and 20mg/kg of FLU. Plasma and MD concentration in the samples were determined by LC-MS/MS and LC-UV.

Results and Conclusion: Multicompartmental models adequately described the pharmacokinetics of VRC and FLU in plasma and tissues. Both drugs showed a good brain's penetration, with Ft equal to 0.71 ± 0.13 and 0.98 ± 0.30 for FLU in healthy and infected animals and 1.07 ± 0.44 and 2.14 ± 1.10 for VRC in healthy and infected animals, respectively. Actual levels reached in the infected and non-infected brains were simulated by MC approach, were the probability of outcomes were predicted based on values of C. neoformans's MIC observed in Brazil (range 0.12-64 ug/mL for FLU and 0.06-0.25 ug/mL for VRC) in 95 clinical and 81 environmental isolates of C. neoformans. The PK/PD index AUC/MIC of 20 for voriconazole and 389 for fluconazole were used as target for success of treatment. The results of MC simulations showed that VRC is more effective against C. neoformans than FLU, for the range of MIC investigated the probability of success with the treatment with VRC was 100% of in healthy or infected tissues, on the other hand for FLU, the outcome was strongly dependent of MIC and infection. FLU attained the probability of 100% of success only for the lowest values of MIC (range 0.12-0.24 ug/mL) and for the higher levels, the probability was only 15%. Based on our results, voriconazole is a good choice to treatment of meningitis associated to C. neoformans due its higher brain's penetration and effectiveness against the microorganism.

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In Vitro Skin Irritation Assay of Medical Devices in the Context of ISO 10993-10

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Introduction: Skin irritation is one of the 3 toxicological endpoints that are always assessed for medical devices. Yet, even if the ISO 10993-10 standard mentions the in vitro OECD test guideline (OECD TG 439), the reference test method remains the in vivo model. The objective of the present work is to present an in vitro protocol on Reconstructed Human Epidermis (SkinEthic RHE) to assess skin irritation of medical devices. SkinEthic RHE model is validated in the TG439 as a full in vitro replacement method to assess the skin irritation of chemicals. Yet, TG439 concern neat chemicals and is not adapted to medical device extracts in which potential leached irritants are diluted in a solvent.

Methods: The proposed protocol is closed to the one developed for cosmetics products with a long exposure time, 24 hours instead of 42 minutes for pure chemical, and no post-incubation (compared to 42 hours for pure chemicals). The benchmark of this protocol has been done according to the publication of Casas et al.1 on polar and apolar solvents spiked with irritants and on different medical devices extracts. The ability of the model to detect low concentration of known irritants spiked at different concentrations in polar and non-polar solvents. The results show dose response toxicity of lactic acid into PBS and of Heptanoic acid into Sesame Oil. The second part of the work presents the results of the test performed onto sample of polymers, PVC and silicone, used for medical devices. Some of these samples, specifically produced for this experiment contain known irritant chemicals. These polymers were prepared according to ISO 10993-12, Biological evaluation of medical devices - Part 12 (Sample preparation and references materials) and extracted in a polar (PBS) and a non-polar (Sesame Oil) solvent.

Results: Cell viability, Il-1a release and histology have been studied 24h after treatment. The results show the ability of this in vitro method to detect low concentration of irritant in solvents used for medical devices extraction. This in vitro method is also able to discriminate between medical devices containing irritant or not. With the set of samples tested, a cut off of 50% of cell viability was sufficient to do the classification. These preliminary data suggest that cell viability alone could be a sufficient biological endpoint to measure for medical devices classification.

Conclusion: The transferability of the protocol has been confirmed with a parallel study in an independent US laboratory. The ongoing confirmation of these results in a round robin study with more samples and more laboratories could lead in the near future to an evolution of the ISO 10993-10 guideline for medical devices to replace the in vivo skin irritation Draize test by in vitro testing on human reconstructed epidermis.

Financial support: This work has been supported by Episkin and L’Oréal Research.
Oral acute toxicity of the oil extracted from the pulp of *Attalea phalerata* Mart. in rats

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**Introduction:** *Attalea phalerata* Mart. ex Spreng., popularly known as “bacuri”, is a native plant from the brazilian Cerrado. It is used in folk medicine as a pulmonary decongestant, an anti-inflammatory for joints and antipyretic. Its composition is high in carotenoids and fatty acids. This study aimed to evaluate the acute toxicity of the oil extracted from *Attalea phalerata* pulp in *Wistar* rats. **Methods:** The experiment was performed in accordance with the OECD protocol (guideline 425) and approved by the Ethics Committee in Animal Experimentation from the UFGD (21/2015). Ten female rats provided from the bioterium of the State University of Maringá were used in this experiment. The animals were housed in polypropylene cages at 23°C, 12h light and dark cycle and had free access to water and food. The oil extracted from the pulp of *Attalea phalerata* (APO) was administered at a dose of 2000 mg/kg/bw, by gavage, to one female. Sequentially, at intervals of 48 hours, the same dose was given to four female rats, totaling five treated animals (APO group 2000 mg/kg/bw). The control group received the vehicle (saline + Tween® 80). Behavior, body weight, water and food intake were recorded daily. All animals were observed for 14 days. After the observation period, the animals were sacrificed by isoflurane anesthesia (inhalation) followed by exsanguination. Target organs (heart, lung, spleen, liver, kidney, uterus and right ovary) were removed, weighed and examined macroscopically. **Results:** The dose of 2000 mg/kg of the APO did not cause any death or changes in the parameters evaluated when compared to the control. There were no statistical changes in body weight gain, food and water consumption. Macroscopic analysis and relative organ weight did not show any changes that indicate signs of toxicity. **Conclusion:** We conclude that the APO LD<sub>50</sub> is higher than 2000 mg/kg. Therefore, it is suggested that the APO has low toxicity. However, studies on chronic toxicity, reproductive toxicity and genotoxicity are necessary in order to proceed to clinical studies of this plant.

Acknowledgments: FUNDECT, CNPq and CAPES.
**Introduction:** There is an increasing incidence of systemic arterial hypertension (HAS) and diabetes mellitus (DM) in Brazil, which makes it possible to find patients with chronic use of antihypertensive and antidiabetic simultaneously, in addition to drugs for co-morbidities control (BRASIL, 2011). Such factors may favor the appearance of Negative Results Associated With Medication (RNM), which have a negative effect on patient health and on health expenses (VARALLO et al, 2013). In view of this, the objective of this study was to verify the presence of RNM concerning the treatment of hypertension and diabetes in patients HIPERDIA Program of the Municipal Health Manaus-AM.

**Methods:** A prospective descriptive quantitative study which was done through a standardized interview with adult patients from August 2015 to April 2016, diagnosed with hypertension and / or DM, registered in the SIS-HIPERDIA in basic health units of the city Manaus-AM. We conducted the measurement of clinical variables to verify the occurrence of RNM based on classifications proposed in the Dader Method of pharmacotherapy follow-up (HERNANDEES, CASTRO and Dader, 2014). After classification, the tabulated data were transcribed for IBM - Statistics® SPSS for analysis. **Results:** The survey was conducted with 289 patients, ensuring a 90% confidence interval, 119 (41,2%) male and 170 (58,8%) female. Applied the Poisson regression model and it was noted that with the significance level of 5% we have strong evidence that the variables: age (p-value: 0,692), gender (p-value: 0,389) and education (p-value: 0,56) did not influence the appearance of MRI in the analyzed sample. Moreover, it was demonstrated that patients with hypertension and concomitant DM were more susceptible to the appearance RNM (p-value: 0,001) compared to the other groups. The incidence of MRI was 1,6 times (60%) higher in patients type 2 diabetes compared to DM patients 1. Patients with HAS and DM showed incidence of MRI increased by 135% compared to patients with type 1 DM alone. The RNM's were identified and classified into three categories: 1) Need - five health problems were identified untreated and do not need medication effect. 2) Effectiveness - higher prevalence of quantitative ineffectiveness. 3) Security - higher prevalence of Insecurity not quantitative. The cause most prevalent found was the non-adherence to treatment (11,8% of patients) usually related to lack of understanding of the importance of non-pharmacological treatment. Thus, the intervention plan best suited to participants recruited for the study was the health education with the alert for lifestyle change measures and the importance of healthy eating and maintaining the practice of regular exercise. **Conclusion:** It was evident the importance of pharmacotherapeutic evaluation of patients with chronic diseases in primary care in order to improve adherence to treatment, improve the quality of life of patients and promote rational use of medicines. There was the need to hold a membership plan adapted to social and family factors where the user is located. Further studies should also be conducted to assess the impact of pharmacotherapeutic follow these patients and to public health in the city of Manaus/AM.

Research approval by the Human or Animal Research Ethical Committee (process number): CAAE: 49066815.0.0000.5020
NEGATIVE OUTCOMES ASSOCIATED WITH MEDICATION IN DIABETIC AND HYPERTENSIVE PATIENTS AS A RESULT OF POOR ADHERENCE TO DRUG TREATMENT

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Introduction: Patients with hypertension (HY) and diabetes mellitus (DM) make concomitant use of many drugs that may favor the occurrence of negative outcomes associated with medication (NOM), a health outcome not consistent with the objectives of pharmacotherapy, usually caused by a drug related problem (DRP). We aimed to identify the occurrence of NOM in patients with hypertension and diabetes in health care units in Manaus/AM. Methods: A descriptive-prospective study of quantitative approach, based on the Dader Method was carried out in basic health units in all districts of the city (East, North, West and South) from August 2015 to April 2016. A semi-structured interview with patients in continuous use of medications was performed. NOM were classified according to need, effectiveness (quantitative and non-quantitative) and security (quantitative, not quantitative). Results: We interviewed 289 patients, 119 men (84.87% with HY and 54.62% with DM) and 170 women (81.7% with HY and 45.88% with DM). It was observed a high rate of patients with only the elementary school degree (32.9%), which reflected the level of their knowledge about the disease. When they were asked about how long they would need treatment, a majority of 52.6% of the patients failed to respond. This also influenced the non-adherence to the treatment, since 11.8% had stopped voluntarily of taking the drugs when they realized improvement in symptoms. For the NOM -quantitative ineffectiveness, it was observed that many patients had high blood pressure and glucose levels, normally associated with noncompliance with treatment when the adverse effects of drugs were perceived or when patients felt better. Such conditions generate low drug levels in the circulation. The non-quantitative ineffectiveness occurred when patients did not achieve the expected results, even taking maximal doses. There was a prevalence of non quantitative insecurity when the security category was assessed. Some patients felt undesirable effects by taking therapeutic low doses of the drug. The NOM quantitative ineffectiveness was observed in cases of multiple treatments, some of them with high doses of the prescribed drugs. This combination resulted in a high risk of side effects as detected: gastric discomfort, dizziness, blurred vision, and weakness. We identified the incidence of three DRP. The most common was non-adherence to treatment, observed 119 times. The patients had forgotten to take their medication on the day of the interview or had stopped performing treatment voluntarily. The second was related to dose/regimen. Here, the prescription was outdated, with low doses or with drug associations more likely to generate adverse effects. The third was linked to the personal characteristics, usually in patients that reported forgetting to take the medication. Conclusion: The level of education is associated with the degree of patient knowledge about their condition. The use of several drugs without proper guidance may cause severe adverse effects. Health professionals working in primary health care must be aware of the symptomatic manifestations of NOM used to treat hypertension and diabetes, as well as be updated about the guidelines to treat such diseases in order to promote greater patient safety and increase the success of treatments. CAAE: 49066815.0.0000.5020
**Study Of Acute Toxicity Of Hpa-05 In Swiss Mice**

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**Introduction:** The search for less toxic drugs resulted in the introduction of synthetic substances in the therapy and its use was disseminated in the twentieth century. HPA-05, 3-phenyl-5-(4-methylphenyl) imidazolidine-2,4-dione is a derivative imidazolidínico. synthetic substance that has shown to have a psicodepressor profile central antinociceptive type. Any substance can be considered a toxic agent, depending on the exposure conditions. This study aimed to evaluate the *in vivo* toxicity of HPA-05, through the study of acute toxicity.

**Methods:** Chemical characterization HPA-05: (200 MHz) - (DMSO-d₆, δ): 2.49 (s, 3H, Ar-CH₃); 5.52 (s, 1H, C5); 7.41–7.71 (m, 9H, aromatic); 9.21 (s, 1H, NH) ppm. (50MHz) – (DMSO-d₆, δ): 21.1 (CH₃); 60.2 (C5); 127.2 (C12:12´); 127.3 (C11:11´); 128.4 (C9); 129.3 (C8:8´); 129.8 (C7:7´); 132.4 (C10); 133.0 (C6); 138.4 (C13); 156.2 (C4); 172.3 (C2) ppm. Acute toxicity study was based on Guide for conducting study nonclinical toxicology and safety pharmacology necessary for the development of drugs and was conducted by treating intraperitoneal in Swiss mice. The animals were divided into two groups with 12 animals (6 females and 6 males). The control group animals received saline. The treated group with HPA-05 it was administered dose of 1000 mg / kg. We assessed consumption of water and feed; weight of the animals; pharmacological screening to detect signs of activity in the central nervous system; haematological and biochemical parameters of blood. After 14 days the animals were euthanized and blood was collected by the brachial plexus. The statistical analysis, we use the test "t" Student unpaired using the software Graph Pad Prism 6.0, and the results were considered significant when presented values of p < 0.05.

**Results:** After 14 days, there weren’t deaths in the groups. The evaluation of behavioral change was evidenced alterations in intervals: 30min, 1h, 2h, 3h and 4h. Animals treated expressed sedation and loss of reflection ear, decreased response to touch and loss reflection of the cornea. There weren’t changes in weight evolution of animals. The water consumption of males increased significantly and there was a decrease for females. There was significant reduction in feed intake for males and females treated. There was statistically significant increase in calcium in females of biochemical parameters. The hematological parameters analysis, occurred reduction of HCM and CHCM, in the treated males. The treated females decreased hematocrit and increased platelets.

**Conclusion:** The present study concludes that after acute treatment with HPA-05 induced low toxicity, permitting others studies about toxicity. This substance have a similar profile of CNS depressant drugs. Legal authorization: Ethics Committee on Animal Research number 2005/13

**Financial Support:** CNPq e CAPES

**References**

EPIDEMIOLOGY PROFILE OF CIPROHEPTADINE INTOXICATION’S ASSISTANCE SERVICE REGISTRED BY CENTRO DE INFORMAÇÕES TOXICOLÓGICAS DO AMAZONAS IN CHILDREN, AGE 0-10 YEARS OLD

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Introduction: Cyproheptadine is a drug that express anti – histaminic (H1 antagonist receptors) and antiserotonergic actions as well anticolinergic proprieties in lower scale. It is classified as first-generation piperidine¹, being used in the medical treatment serotonergic syndrome ², nightmares caused by post-traumatic stress disorder³ and associated with multivitamins for children for hunger stimulus⁴. In Brazil, the substance has been sold at pharmacies, usually added to vitamin complexes. The aim of this study was to establish the profile of poisoning among children aged 0-10 that were reported to the Centro de Informações Toxicológicas do Amazonas (CIT-AM), in Manaus, Amazonas, Brazil.

Methods: A descriptive and cross-section study, made by the screening and selection of cases recorded by CIT-AM, a public service open 24 hours, by telephone, in Manaus – Amazonas. Those call records were searched at DATATOX, it’s database. It were included cases of medicine intoxication registered between January 2014 and May 2016. The selected cases were analysed utilizing Excel© program and the percentage of records related to children between 0 and 10 years, cyproheptadine intoxications in the children population and the symptoms commonly associated were calculated.

Results: Between January 2014 and May 2016, 2193 assistance services for exposure and intoxication with several types of substances were realized. Of those, 970 (44.2%) were drug-related. Children, aged 0 to 10 years, compound to the most of patients attended, amounting to 523 (53%) cases.

Cyproheptadine represented 11.6% of cases, totalling 61 calls involving intoxication or appearance of side effects after the therapeutic dose in children. On call, patients have related experiencing the following symptoms, in order of frequency: agitation (54.09%), hallucinations (16.39%), lethargy (11.47%), confusion (8.19%) and aggressiveness (6.56%). In all cases involving the drug, it was associated with multivitamins complex.

Conclusion Most patients between ages 0 to 10 years old, after exposure to cyproheptadine, presented agitation, hallucinations and lethargy. This, in vitamin complexes, was responsible for all intoxication cases analysed. It is important to note that use of vitamin complexes is common and of easy access to the population. These results show that it a recurrent situation, and more studies about the possible side effects caused by cyproheptadine, as well symptoms in intoxication cases would be relevant.

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References

EVALUATION OF GENOTOXICITY OF BIODEGRADABLE NANOCAPSULES

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Introduction: The fast growth of nanotechnology has contributed to the emergence of a new area of knowledge, the nanotoxicology, which has been studying the security of nanomaterials, especially in the face of human exposures. Additionally, the biodegradable polymeric nanocapsules have been demonstrated as potential carriers for drugs for the treatment of various pathologies. The aim of this study was to evaluate the potential genotoxicity of biodegradable lipid-core nanocapsules (LNC) containing poly (ε-caprolactone). Methods: Male wistar rats (n=8/group), aged 6-8 weeks, were anesthetized and received 10 ml/kg LNC (LNC group), saline (control group) and 38 mg/dl tween solution in glycerol, intravenously at a flow of 2 ml/h. The euthanasia was performed 14 days after administration, whole blood were collected in heparin tubes, for performing the alkaline comet assay and the femur was removed for micronucleus assay. For comet assay, 100 cells were counted in a fluorescence microscopy (40x objective) and the % DNA in tail was measured through the CometScore software. The results were expressed as mean ± standard deviation. Values of p<0.05 were considered significant. This project was approved by Ethics Committee from Hospital de Clínicas de Porto Alegre (HCPA) register nº130279. Results: The % DNA in tail for control, glycerol and LNC groups were 8.12%, 6.25% and 8.04%, respectively. There was no significant difference between groups (p>0.05, ANOVA). Through these results we verified that there were no breaks in the DNA in the cells analyzed. Conclusion: The results demonstrated that biodegradable nanocapsules containing poly (ε-caprolactone) examined in this work are not genotoxic. The lack of DNA damage indicate the security of the use of this nanocarriers. Acknowledgements: This work was supported by FAPERGS, CNPQ and FIPE-HCPA.
Assessment of Toxicity of Benzo(b)fluoranthene Present in Asphalt Fumes in the Nematode *Caenorhabditis elegans*

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**Introduction:** Asphalt is a complex mixture containing many organic compounds. Emissions of asphalt fumes, generated after heating, may contain polycyclic aromatic hydrocarbons (PAHs). *In vitro* and *in vivo* studies are proposed to evaluate and elucidate the toxicity of the substances present in the fumes. The alternative model *C. elegans* has been of great importance to assess efficacy and toxicity of many xenobiotics. The purpose of this study was to evaluate the toxicity of benzo(b)fluoranthene.

**Materials and Methods:** *C. elegans* strain N2 (wild-type) was originally obtained from the *Caenorhabditis Genetics Center* (CGC) and was maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 as a food source and kept at 20ºC in a BOD incubator. The synchronization of the worms was made to obtain the eggs and L1 worms. For the treatment of the nematodes, the tested concentrations of benzo(b)fluoranthene ranged from 50 to 300 mg/L. Survival assays with 2500 worms were made to determine the lethal dose (LD₅₀) of the molecule. The *C. elegans* is an invertebrate nematode, therefore there is no need of approval in the ethics committee (Goldim, J.R; Oliveira, E.M; 2014). Statistical analysis of significance was carried out by one-way ANOVA followed by Tukey post-hoc test. The results were expressed as mean ± standard deviation (SD). Values of $p \leq 0.05$ were considered significant.

**Results:** The LD₅₀ found to benzo(b)fluoranthene was 219.20 mg/L. Similar to our findings, Tae-Hoon Nam et al. (2015) reported in *Eisenia fetida* a LD₅₀ of 300 mg/L to fluorene, another PAH found in asphalt fumes.

**Conclusions:** Our findings demonstrated that benzo(b)fluoranthene presented relevant toxicity in the alternative *in vivo* model *C. elegans*. The use of a multicellular organism is considered a good alternative to evaluate the toxicity of PAHs and this is an important point once these PAHs are present in the environment, and the knowledge regarding the toxic effects of these pollutants are necessary for potential risk evaluation.

**Acknowledgements:** Petrobras and CNPq/Brazil.
11.020 Bioequivalence study of two formulations of Enalapril 10 mg tablets in healthy volunteers of both sexes under fasting conditions. Lima MCN, Lemos APD, Pontes AV, Souza ACC, Leite ALAS, Nascimento DF, Frota Bezerra FA, Moraes MO, Moraes MEA. UNIFAC-UFC

Introduction: Enalapril is a Angiotensin Converting Enzyme (ACE) inhibitor drug which is used for the treatment of essential renal hypertension, left ventricular dysfunction and congestive heart failure. The aim of this study was to evaluate the bioequivalence between two formulations Enalapril maleate 10mg in healthy volunteers of both sexes under fasting conditions. Methods: Twenty-four volunteers aged between 18 and 50 years and BMI between 18.5 – 28.7 kg/m² were selected for the study after clinical evaluation (physical examination, ECG) and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, albumin and total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts and routine urinalysis. All subjects were negative for HIV, HCV and HBV serology. The Ethics Committee of the Federal University of Ceara approved the clinical protocol (Protocol n° 751332), and all subjects gave their written informed consent. This study was open, randomized, two treatments, two-period crossover design, during which the volunteers received enalapril 10mg (Test formulation) or enalapril 10mg (Renitec ® - MERCK Sharp & Dohme Farmaceutica Ltda as Reference formulation) with a three days washout period. Enalapril concentrations were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC-MS-MS) with positive ion electrospray ionization using selected daughter ion monitoring (MRM). The formulations were considered bioequivalent if the 90% Confidence interval for the log-transformed values were within the predetermined equivalence range of 80%–125% for Maximum concentration (C_{max}) and Area under the curve (AUC), according to the guidelines of the Brazilian Health Surveillance Agency (ANVISA). The pharmacokinetics parameters are showed as geometric mean. Results and Discussions: The method validation investigated the parameters recommended for the bioanalytical methods and got good results with limit of quantification of 2ng/mL. The retention time to enalapril was 1.50 minutes and to lisinopril (internal standard) was 0.79 min. The response was linear in the concentration range of 2-300 ng/mL (r=0.9959). The confidence interval (IC) for all parameters required to evaluate the bioequivalence were within the range of 80-125% as established by the National Health Surveillance Agency (ANVISA) and the Food and Drug Administration Agency (FDA). The geometric mean ratios (test:reference) for AUC_{0-\text{t}}, AUC_{0-\text{inf}} and C_{\text{max}} were 105.87% (90% CI, 95.92–104.21), 110.75% (90% CI, 95.88 – 103.27) and 60.65% (90% CI, 98.58 – 111.45), respectively. Thus, this study concluded that the Enalapril test formulation is bioequivalent in terms of both rate and extent of absorption to reference formulation of Enalapril (Renitec®), when administered in healthy volunteers. Financial support: CNPq, InCB, MS-RNPC-UNIFAC-HM, FINEP. Process number approved by Ethics Committee of the Federal University of Ceara: n° 751332.
11.021 Analysis of the pathogenicity factors of *Sporothrix pallida* to identification targets for drug design. Sastre IS¹, Cabrera OG², Nascimento LC³, Tiburcio RA², José J², Beretta ALRZ¹ ¹Centro Universitário Hermínio Ometto – UNIARARAS – Microbiology, ²IB-Unicamp – Genômica e Expressão Genética, Evolução e Bioagentes, ³Unicamp – Processamento de Alto Desempenho

The genus *Sporothrix* includes at least four pathogenic species of human and animals causing sporotrichosis: *S. schenckii* sensu stricto, *S. brasiliensis*, *S. globosa*, and *S. luriei*. *S. pallida*, known as non-pathogenic species, was identified as the causal agent of keratitis in humans. The fact that *S. pallida* have gained a new host and have shown pathogenic capacity motivated the development of this study. The work focused on the analysis of pathogenicity factors potentially produced by *S. pallida* and identification of candidates for drug design. It was performed gene prediction of the *S. pallida* genome resulting in 10,834 genes. Secretome prediction identified 559 (5%) polypeptides. Functional annotation of the secretome showed that 11% and 46% have no-similarity (No-hits) to proteins in NR and Swiss-Prot, respectively. These proteins can be specific to *S. pallida* or *Sporothrix* genera. Functional annotation of the remainder secretome (43%) showed enrichment proteins with hydrolase activity, oxidoreductase activity and ion binding function. Analysis of DbCAN showed reduction in the number of proteins with carbohydrate activity compared to *S. brasiliensis* and *S. schenckii* suggesting differences in the colonization mechanisms. Also, were identified potential pathogenicity factors in *S. pallida* total genome by comparison with a Data Bank of Host-Pathogen Interaction (PHI). As the results, 2,720 proteins from *S. pallida* were identified with counterparts in PHI. Of this total, 1,104 proteins and 208 proteins belong to the categories related with reduction and loss of pathogenicity, respectively. In these groups were founded proteins related with transport of nutrients, detoxification of compounds, chitin degradation, etc. Finally, were identified 24 candidates as target for drug design. The most representative category in this group was transporters involved in efflux of toxins. This work provides tools for understanding the host plasticity of *S. pallida* and possible mechanisms for its control. Keywords: *Sporothrix*, *S. pallida*, genome, secretome, pathogenicity factors, effectors.
11.022 Enoxaparin does not modulate pharmacokinetics of digoxin in patients with heart failure. Souza FC, Alvim-Silva T, Scaramello CBV UFF – Fisiologia e Farmacologia

Introduction: Digoxin is commonly prescribed at a daily dose of 0.125 or 0.25 mg orally. Due to its narrow therapeutic range (0.8 to 2.0 ng/mL), digoxin requires constant plasma concentration monitoring. Intoxication may occur due to drug interactions or health status that change digoxin pharmacokinetics parameters and can be avoided by the adjustment of digitalis therapeutic regimen (Souza et al., Int J Cardiol, 179:343, 2015). The aim of the present work was to study the effect of enoxaparin, an anticoagulant, on digoxin pharmacokinetics. Methods: Patients admitted to the cardiomyopathy ward of Instituto Nacional de Cardiologia (INC) at Rio de Janeiro (Brazil) were recruited through a pharmacotherapeutic screening. Eligibility criteria involved (1) male patients using digoxin 0.125–0.250 mg p.o. (8:00 am) during at least 5 days, (2) aged between 21 and 60 years-old, and (3) New York Heart Association (NYHA) Class III. Eligible patients provided a consent form. Enrollment was stratified into two groups on the basis of the current adherence of patients to digoxin therapy alone (control– enoxaparin naïve, n=10) or in combination with daily enoxaparin 20 mg s.c. (8:00 am) administration during at least 3 days (enoxaparin, n=8). Blood samples were collected at 6 different points along 24 h after digoxin oral administration. Measurements of digitalis plasma concentration were performed using immuno-chemiluminescence method (Souza et al., Int J Cardiol, 179:343, 2015). Pharmacokinetics parameters were determined for each group- Area Under the Curve (AUC), calculated by the linear trapezoidal rule; Maximum plasma Concentration (C_{max}); Time to C_{max} observation (T_{max}) and the ratio CL/F (clearance of the drug in plasma, per unit time, in accordance with its bioavailability). GraphPad Prism Software Inc. (San Diego, CA), version 5.0, was used for graphic representation, pharmacokinetics parameter determination and statistical analysis. Data are presented as mean ± SEM. A two-tailed unpaired Student’s t-test was performed to identify significant between-group differences in normally distributed variables. Statistical significance was accepted at the 0.05 level. Results: Groups presented comparable characteristics (Control– age=48.8±4.3years, weight=73.1±3.8kg, height=1.74±0.03m, creatinine clearance=71.5±6.3mL/min; Enoxaparin– age=46.5±3.9years, weight=64.4±6.5kg, height=1.64±0.07m, creatinine clearance=71.5±6.3mL/min). There was no statistical difference between groups about digoxin pharmacokinetics parameters due to enoxaparin association (Control– AUC_{0-24h}=24.55±2.54ng.h/mL, C_{max}=1.35±0.14ng/mL, T_{max}=2.23±0.72h, CL/F=0.112±0.028L/h/kg; Enoxaparin– AUC_{0-24h}=24.62±3.00ng.h/mL, C_{max}=1.44±0.16ng/mL, T_{max}=2.38±0.82h, CL/F=0.158±0.036L/h/kg). Conclusions: Our findings suggest that enoxaparin therapy does not modulate digoxin pharmacokinetics in patients with heart failure. In this context, this anticoagulant and digoxin may be administered concomitantly without the need for dose adjustment of the digitalis. Financial Support: FAPERJ, CAPES, CNPq, PROPPI/UFF. INC Ethics Committee on Human Research approval 0306/07-12-10.
Impact of genetic polymorphisms related to asymmetrical dimethylarginine metabolism on sildenafil responsiveness in erectile dysfunction. Milanez-Azevedo AM\textsuperscript{1}, Viana-Figaro F\textsuperscript{2}, Molina CAF\textsuperscript{3}, Andrade MF\textsuperscript{4}, Muniz JJ\textsuperscript{2}, Tanus-Santos JE\textsuperscript{1}, Tucci Jr S\textsuperscript{3}, Lacchini R\textsuperscript{2} FMRP-USP – Farmacologia, \textsuperscript{2}EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas, \textsuperscript{3}FMRP-USP – Cirurgia e Anatomia, \textsuperscript{4}FMRP-USP – Cirurgia, Ortopedia e Traumatologia

Introduction: Erectile dysfunction (ED) is a disease related to deficient nitric oxide (NO) signaling. NO is produced from L-arginine by three nitric oxide synthases (NOS). Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of all NOS, and may be a plasma marker of endothelial function and a risk factor for cardiovascular disease. ADMA is mainly metabolized by the enzymes dimethylarginine dimethylaminohydrolase 1 and 2 (DDAH1 and DDAH2). Several studies have associated alterations in genes, expression or activity of DDAH enzymes with disorders in with impaired NO signaling. This study aims to evaluate if genetic polymorphisms in DDAH1 and DDAH2 genes are associated with Sildenafil responsiveness in ED.

Methods: Were included sixty eight post-prostatectomy (PED) and sixty five clinical ED (CED) patients from the Urology Clinic of the University Hospital of the Faculty of Medicine of Ribeirao Preto, whose samples were collected in previous studies. Erectile function was evaluated using the five-item version of International Index for Erectile Function (5-IIEF) questionnaire. We assessed the patients' responses to Sildenafil by subtracting the 5-IIEF score before treatment from the 5-IIEF score after treatment (ΔIIEF). The patients in the two groups were classified as good responders (GR) or poor responders (PR) if their percentage of maximum possible response (%MPR) was higher or lower than the median, respectively. The genotyping of rs1554597 DDAH1 gene polymorphism was determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The statistical analyses were made by: Kruskal-Wallis and contingency tables. Results: We have found that the genotype frequency of the rs1554597 polymorphism in PED group was: TT (44%), TC (43%), CC (13%), which was not different significantly from European Caucasians (CEU; P=0.419), but was different from Yoruba (YRI; P=0.027). Regarding CED group, genotype frequency was: TT (49%), TC (42%) and CC (9%), which was not different from both CEU and YRI (all P>0.05, not shown). This polymorphism was not associated with changes in ΔIIEF scores nor %MPR (all P>0.05, not shown). However, the genotypes distributions were different between GR and PR within PED group (P=0.004). The CC genotype was associated with GR (P=0.011; OR=0.29; 95% CI=0.11 to 0.78). We haven't found any other significant associations. These are preliminary results, as we will test another DDAH1 polymorphism and other two DDAH2 polymorphisms, and the sample size will likely increase with time. Conclusion: CC genotype of rs1554597 is associated with a better outcome of Sildenafil erectile dysfunction therapy in patients that undergone prostatectomy surgery. Financial support: CNPq, FAPESP, CAPES. Approval at the Human Research Ethics Committee: CAAE 51398915.1.0000.5393
Introduction: The science of pharmacogenomics has advanced significantly in the last five years, but it is still in infancy and is mostly used on research basis. The Pharmacogenomics helps identify interindividual variabilities in drug response (both toxicity and effectiveness). This information will make it possible to individualize therapy with the intent of maximizing effectiveness and minimizing risk. Objectives. The aims of this work are to present the bases of pharmacogenetic, the existing international and national regulations related with this topic and to show the proposal of Cuban Guidance about Pharmacogenetic. Methods: It was done a revision in the international literature about the existing regulations, guidance, concept paper etc, and the national regulations related with this topic will be shown. Results: In this section it is shown, the increase of biological product registration and authorized clinical trials in Cuba and the necessity for carrying out this kind of studies for our products. We will show the main biomarkers for pharmacogenetics studies and a Cuban general guidance for submission of this type of research. It is divided in 4 sections, Introduction, objectives and scope, terms and definition, regulatory recommendations (reception, codification and sample storage biomarkers, ethical considerations). Conclusion: This Cuban guidance will be considered the first guidance related with pharmacogenomic in Latin America. The hope for the future is that through personalized medicine, doctors and patients will be able to make better-informed choices about treatment. This treatment will avoid the adverse drug reaction to the medication and will improve the diagnosis diseases as well as the prevention and treatment of diseases.

Introduction: Cardiovascular diseases are common causes of death. Prevention and optimal management of acute coronary syndrome (ACS) can reduce this mortality. It was intended to determine the pharmacological measures implemented as part of secondary prevention in patients who suffered an ACS to identify whether the management established is part of the current recommendations and intervening cases with incomplete treatment to ensure that all pharmacological measures are established with the benefit proven in such patients. Methods: Quasi-experimental study, before and after in patients who suffered an ACS, affiliated with the Colombia health system between 1 January and 31 December 2014. Each case was followed up a year from the time of occurrence of SCA and the researchers reviewed all medication dispensed. Socio-demographic, clinical, and pharmacological variables were considered. From the information of who was missing some prescription (β-blockers + inhibitors renin angiotensin aldosterone system + dual antiaggregation + statin) according to the criteria Screening Tool to Alert doctors to Right Treatment (START) an intervention on doctors performed showing analysis of each case, the missing medicine and evidence supporting the recommendation. Three months later the results were measured. Results: A total of 829 patients with ACS undergoing percutaneous coronary intervention (90.1%) or coronary bypass (9.9%) in 16 different cities were found. The mean age was 63.8 ± 10.6 years, and 73.1% were men. Recommended by international guidelines drug therapy in these patients was fulfilled in 729 patients (87.9% of cases). About the remaining 100 patients an intervention was made to get them to prescribe the drug missing which was achieved in 23.0% of cases. Statistical analysis showed no significant differences according to drug group that should be initiated or the success of the intervention. Conclusion: Most patients who suffered an ACS are adequately treated after percutaneous intervention or coronary bypass, with those recommended by clinical practice guidelines medicines. Limited success in adjusting therapy was achieved following the recommendations given to the physicians responsible. Financial support information: Universidad Tecnológica de Pereira, Audifarma S.A. The Bioethics Committee of the Universidad Tecnológica of Pereira (Pereira, Colombia) reviewed and approved the research as ‘research without risk’ and guaranteed the anonymity of the patients, following the Declaration of Helsinki. References: 1.Guía de práctica clínica para el Síndrome Coronario Agudo. Sistema General de Seguridad Social en Salud. Bogotá, Colombia: Ministerio de Salud y Protección Social.; 2013. 2.Smith SC, J Am Coll Cardiol. 2011;58:2432 3.Gallagher P Int J Clin Pharmacol Ther. 2008;46:72
CYP1A2*1F polymorphism influences the response to clozapine treatment.
Ghedini PC, de Brito RB UFG – Farmacologia

Introduction: Clozapine (CLZ) is an atypical antipsychotic of choice for patients having treatment refractory schizophrenia (TRS). However, approximately 30% of TRS patients do not fully respond to CLZ treatment, and such patients constitute the group known as clozapine non-responders or super-refractory schizophrenics (SRS). Inter-individual variation in response to antipsychotics may be associated with genetic factors, as polymorphisms present in the cytochrome P450 (CYP) drug-metabolizing enzymes. Knowing the CYP1A2 is the main enzyme involved in the CLZ metabolism and the CYP1A2 *1F (−163C>A, rs762551) polymorphism is associated with an increase in enzyme function, this study investigated the influence of CYP1A2 *1F in TRS and SRS in a naturalistic clinical setting.

Methods: CYP1A2*1F genotype testing included 54 individuals, 27 (50%) of whom were classified as refractory and 27 (50%) as super refractory. A group of 64 healthy volunteers of both genders was included in the genotype testing. The psychopathological data of each patient was defined according the Brief Psychiatric Rating Scale-anchored version (BPRS). DNA was extracted of venous blood and the polymorphism was analyzed by polymerase chain reaction (PCR) and sequencing technique. The phenotype was estimated based on the combination of alleles detected. Group comparisons were performed, using $X^2$ and Fisher’s exact test. A p-value $< 0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS software package (version 21.0; SPSS Inc., IL, US).

Results: The genotype testing for CYP1A2*1F showed that genotype frequencies of TRS patients, SRS patients, and healthy individuals were, respectively: 7 (26%), 0 (0%), and 16 (25%) for homozygous wild-type (*1A/*1A); 11 (40.7%), 7 (26.0%), and 11 (34.4%) for heterozygous (*1A/*1F); and 9 (33.3%), 20 (74%), and 13 (40.6%) for homozygous mutant (*1F/*1F). The allele frequencies observed for TRS patients, SRS patients, and healthy individuals were, respectively: 46.3%, 13.0%, and 42.2 for wild-type allele C; and 53.7%, 87.0%, and 57.8% for mutant allele A. The analysis of the severity of symptoms by BPRS showed that carriers of genotype *1F/*1F had the highest scores before and after treatment compared to the other genotypes ($P= 0.010$ and $P= 0.002$, respectively). It was observed that 100% of SRS patients are carriers of the allele *1F (*1A/*1F or *1F/*1F), and a significant correlation was observed between super-refractoriness and genotype *1F/*1F ($P=0.0002$).

Conclusion: The results suggest the CYP1A2*1F polymorphism is associated with super-refractory schizophrenia. Financial support: Fapeg, Capes, CNPq Research approved by the Human Research Ethical Committee from Federal University of Goiás (protocol number 1.483.734).